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A COMPARISON OF THE AMINO-TERMINAL SEQUENCE OF THE L7/L12-TYPE PROTEINS OF *ARTEMIA SALINA* AND *SACCHAROMYCES CEREVISIAE*

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1. Introduction

The ribosomal protein L7/L12 functions in the GTP-dependent reactions of protein synthesis [1,2]. A comparison of its primary structure, both in procaryotic and eucaryotic organisms, would be of great interest, not only for localizing active sites involved in protein biosynthesis, but also for tracing evolutionary changes of the protein moiety of the ribosome.

Up to now, only the complete primary structure of *Escherichia coli* L7/L12 protein has been published [3]. Visentin et al. [4] performed sequence studies of the amino-terminal region of the *Bacillus stearothermophilus* protein, and they found that the first 15 residues are highly homologous with residues 2-16 of the *E. coli* protein. Oda et al. [5] published partial sequences of an acidic protein, L20, of *Halobacterium cutirubrum* which appears to be homologous to L7/L12. The amino-terminal region of this protein shows a substantial degree of homology with the region, starting at residue 35 of the *E. coli* protein. The presence of acidic proteins in 60 S ribosomes of the brine shrimp *Artemia salina* was previously reported by Möller et al. [6]. These proteins were shown to function in the GTP-dependent reactions in ribosomes [6,7]. We therefore assume that these proteins are equivalent to bacterial L7/L12 and we hereafter call them L7/L12-type proteins.

The present communication is concerned with the comparison of the aminoterminal region of the L7/L12-type proteins from *Artemia salina* and the yeast *Saccharomyces cerevisiae*. The results indicate a remarkable conservation of this region and a clear relationship to the aminoterminal part of the protein from the halophile.

2. Materials and methods

The 80 S ribosomes from *A. salina* cysts were prepared as described earlier [6]. The L7/L12-type proteins were extracted from 80 S ribosomes by the procedure of Hamel et al. [8] and purified by carboxymethylcellulose and diethylaminoethyl-cellulose chromatography, as will be described elsewhere.

The yeast L7/L12-type proteins were extracted from 80 S or 60 S ribosomes and purified by diethylaminoethyl-cellulose chromatography, as will be described in detail elsewhere.

2.1. *Sequence determination of* Artemia *protein*

2.1.1. Automated solid-phase Edman degradation

Various methods were applied to couple the intact protein to suitable matrices. The best results were

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obtained by coupling the protein (300 nmol) from solutions containing 6 M guanidinium chloride to *p*-phenylenediisothiocyanate-treated aminopropyl glass (cf. Wachter et al. [9]). 30% of the protein was covalently bound to the matrix. Solid-phase Edman degradation was performed essentially as described by Laursen [10] on a Sequemat peptide sequencer. Identification of the phenylthiohydantoin amino acids was done by thin-layer chromatography on silicagel and by amino acid analysis after back-hydrolysis in HI, as described by Smithies et al. [11]. Confirmation of the sequence found was obtained by solid-phase sequencing of a highly hydrophobic peptide, comprising residues 3–25; this peptide was isolated from tryptic digests of the whole protein after peptide mapping.

2.1.2. Automated liquid-phase Edman degradation

The amino-terminal part of the intact protein (350 nmol) was sequenced on a Beckman Model 890C protein sequencer. The *N,N*-dimethylbenzylamine program was used [12]. Identification of the phenylthiohydantoin amino acids was done by thin-layer chromatography, by gas chromatography after trimethylsililation [13] and by high-voltage electrophoresis.

2.1.3. Manual Dansyl-Edman degradation

This was performed on a nanomole scale on the intact protein, using the method of Weiner et al. [14]. The first 6 residues, with the exception of Arg-2, were identified.

2.2. *Sequence determination of yeast protein*

The yeast protein (ca. 200 nmol) was subjected to automated Edman degradation on a Beckman Model 890 sequencer equipped with an undercut reaction cup and N_2-flush system, with 1 M Quadrol buffer program. Identification of the phenylthiohydantoin amino acids was performed with a Beckman GC-45 gas chromatograph with SP 400, directly and also after silylation with *N,O*-bis (trimethylsilyl)acetamide [13]. All the phenylthiohydantoin amino acids were also identified as free amino acids after hydrolysis with HI (ref. [11]).

3. Results and discussion

Figure 1 shows the amino-terminal region of *A. salina* 17/L12-type protein and the corresponding protein from *S. cerevisiae*, together with the sequences of procaryotic *E. coli* L7/L12 and *H. cutirubrum* L20.

	5							10				
A. salina	Met	Arg	Tyr	Val	Ala	Ala	Tyr	Leu	Leu	Ala	Ala	Leu
S. cerevisiae	Met	Lys	Tyr	Leu	Ala	Ala	Tyr	Leu	Leu	Leu	Asx*	Glx*
H., cutirubrum	Met	Glu	Tyr	Val	Tyr	Ala	Ala	Leu	Ile	Leu	Asn	Glu
E. coli	Ala	Ala	Ala	Val	Ala	Val	Ala	Ala	Gly	Pro	Val	Glu
	35				40					45		

		15					20					25
A. salina	Thr	Gly	Asn	Ala	Asp	Pro	Thr		Ala	Asp	Ile	Gly	Lys
S. cerevisiae	Gly	Gly	Asx	Ala									
H. cutirubrum	Ala	Asp	Glu	o	Glu	Leu	Thr	Glu	Asp	Asp	Ile	Thr	Gly
E. coli	Ala	Ala	Glu	o	Glu	Lys	Thr	Glu	Phe	Asp	Val	o	o
					50					55			

Fig.1. The aminoterminal region of 50 S/60 S acidic L7/L12-type ribosomal proteins. The data of the *H. cutirubrum* protein and the *E. coli* protein have been taken from ref. [3] and [5], respectively. *Tentative identification. Asp-21 should be Asn-21.

Comparison of the two eucaryotic sequences shows a remarkable degree of homology in this part of the protein molecule: 9–10 out of 16 residues are homologous and several of the other ones may be related through single-base point mutations. There exists also a homology with *H. cutirubrum* L20: 5–7 out of 16 residues of the yeast protein, and 8 out of 25 residues of the *A. salina* protein are homologous in this part of the polypeptide chains. The clustering of two tyrosine residues near the amino-terminal end is noteworthy as is the occurrence of hydrophobic residues in equivalent positions of the polypeptide chains. A homology between *H. cutirubrum* L20 and *E. coli* L7/L12 was reported earlier [5], if the two chains were aligned such that residue-35 of *E. coli* L7/L12 matched the aminoterminal methionine residue of *H. cutirubrum* L20: 9 out of 25 residues were homologous in this case. On the basis of its aminoterminal sequence there is no doubt that *H. cutirubrum* resembles a eucaryotic rather than a procaryotic organism. In this connection we like to mention the occurrence of a bacteriorhodopsin in the membrane of the halophile, and the fact that the biosynthesis of the proteins in this organism is initiated by Met-tRNA rather than fMet-tRNA (S. T. Bayley, personal communication).

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